

Prof. Dr. med. H.-P. Werner  
Specialist for Hygiene

**HYG  
CEN**

Centrum für Hygiene  
und medizinische Produktsicherheit  
(Center for Hygiene  
and Product Safety)

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Antiseptica  
chem.-pharm. Produkte GmbH  
Carl-Friedrich-Gauss-Straße 7  
D-50259 Pulheim/Brauweiler

03.07.1996  
Prof. We/gr

MANORAPID® SYNERGY  
Hygienic hand disinfection

### EXPERT OPINION

Your order: 01.06.1996

Characteristics of the product:

Active ingredients as specified  
by manufacturer: 54.1 % (100 %) ethanol  
10.0 % (100 %) n-propanol

Odor: alcoholic

Appearance of product: clear, colorless liquid

Batch number: VP 100/5

pH values (glass electrode): concentrated: 3.5  
50 % in WSH 3.8

Prof. Dr. med. H.-P. Werner  
c/o HygCen • Centrum für Hygiene  
und medizinische Produktsicherheit GmbH

Bornhövedstraße 78  
D-19055 Schwerin

Tel: + 49 385 - 56 82 65  
Fax: +49 385 - 56 82 67

Methodology:

## Preliminary tests:

"Guidelines for testing and evaluation of chemical disinfection processes" (dated: 01.01.1981) and "Testing and evaluation of chemical disinfection processes" (dated: 12.07.1991) of the German Society for Hygiene and Microbiology.

## Tests under everyday conditions:

Hygienic hand disinfection as per EN 1500

Escherichia coli NTCC 10538

Test Organisms:

Staphylococcus aureus	ATCC 6538
Escherichia coli	ATCC 11229
Proteus mirabilis	ATCC 14153
Pseudomonas aeruginosa	ATCC 15442
Candida albicans	ATCC 10231

**Determination of the bacteriostatic and fungistatic efficacy as well as of appropriate inactivation substances**

(based on the "guidelines" I/2. 1)

Growth inhibition after 72 hours of incubation at 37°C

	Final concentrations of MANORAPID SYNERGY					
	15	10	7.5	5.0	4.0	3.0
<b>No inactivation additive</b>						
S. aureus	-	-	-	+	+	+
E. coli	-	-	-	-	+	+
P. mirabilis	-	-	-	+	+	+
P. aeruginosa	-	-	-	-	+	+
C. albicans	-	-	-	+	+	+
<b>3 % Tween 80 + 0.3 % lecithin + 0.1 % cysteine</b>						
S. aureus	-	-	-	+	+	+
E. coli	-	-	-	-	+	+
P. mirabilis	-	-	-	+	+	+
P. aeruginosa	-	-	-	-	+	+
C. albicans	-	-	-	+	+	+
<b>3 % Tween 80 + 3 % saponin + 0.1 % histidine + 0.1 % cysteine</b>						
S. aureus	-	-	-	+	+	
E. coli	-	-	-	-	+	
P. mirabilis	-	-	-	+	+	+
P. aeruginosa	-	-	-	+	+	+
C. albicans	-	-	-	+	+	+
<b>3 % Tween 80 + 0.3 % lecithin + 0.1 % histidine + 0.5 % sodium thiosulfate</b>						
S. aureus	-	-	-	+	+	+
E. coli	-	-	-	-	+	+
P. mirabilis	-	-	-	+	+	+
P. aeruginosa	-	-	-	-	+	+
C. albicans	-	-	-	+	+	+

Inactivation combination in further tests:

**3 % Tween 80 + 3 % saponin + 0.1% histidine + 0.1 % cysteine**

**Key to symbols:**

+ = turbidity due to microbial replication

- = no turbidity following microbial replication

Bactericidal and fungicidal action in the qualitative suspension test ((based on the "Guidelines" I/2.2)

To enable comparison, these tests were conducted in parallel. We used the elucidated inactivation combination and the aforementioned ATCC strains. Subcultures were incubated at 37°C for 7 days.

Unchallenged test

Test organism (log. cfu/ml)	Con. (%) of MANORAPID SYNERGY	Exposure Time (min)			
		1/2	1	2	5
<hr/>					
S. aureus	(9.37)				
100		-	-	-	-
50		-	-	-	-
25		+	+	+	+
5		+	+	+	+
<hr/>					
E. coli	(9.25)				
100		-	-	-	-
50		-	-	-	-
25		+	+	-	-
5		+	+	+	+
<hr/>					
P. mirabilis	(9.48)				
100		-	-	-	-
50		-	-	-	-
25		+	+	-	-
5		+	+	+	+
<hr/>					
P. aeruginosa	(9.56)				
100		-	-	-	-
50		-	-	-	-
25		+	+	-	-
5		+	+	+	+
<hr/>					
C. albicans	(8.05)				
100		-	-	-	-
50		-	-	-	-
25		+	+	+	+
5		+	+	+	+

The test organisms were obtained from inoculation of cultures with growth. Reinoculation of cultures without growth led to replication after 24-h incubation.

Key to symbols:

+ = growth

- = no growth

**Bactericidal action in the quantitative suspension test**  
(based on the "Guidelines" I/2.3)

To enable comparison, the tests were conducted in parallel. The room temperature was 24°C.

Microbial content of the baseline suspension:

S. aureus	ATCC 6538:	9.24 log/ml
P. aeruginosa	ATCC 15442:	9.80 log/ml

Test organisms Concentrations (%) of MANORAPID SYNERGY	Reduction factors (log) after exposure time in minutes			
	1/2	1	2	5
<b>No albumin challenge (I/2.3.1)</b>				
<b>S. aureus</b>				
100	≥5.21	≥5.20	≥5.18	≥5.08
50	≥5.21	≥5.20	≥5.18	≥5.08
25	0.77	0.85	1.77	4.78
5	0.40	0.49	0.49	0.60
Control value (log) in WSH	6.21	6.20	6.18	6.08
<b>P. aeruginosa</b>				
100	≥5.34	≥5.32	≥5.30	≥5.24
50	≥5.34	≥5.32	≥5.30	≥5.24
25	≥5.34	≥5.32	≥5.30	≥5.24
5	0.09	0.07	0.24	0.33
Control value (log) in WSH	6.34	6.32	6.30	6.24
<b>With 0.2% albumin challenge (I/2.3.2)</b>				
<b>S. aureus</b>				
100	≥5.32	≥5.28	≥5.21	≥5.04
50	≥5.32	≥5.28	≥5.21	≥5.04
25	0.63	0.79	0.96	2.28
5	0.61	0.65	0.65	0.72
Control value (log) in WSH	6.32	6.28	6.21	6.04
<b>P. aeruginosa</b>				
100	≥5.33	≥5.30	≥5.13	≥5.10
50	≥5.33	≥5.30	≥5.13	≥5.10
25	3.31	≥5.30	≥5.13	≥5.10
5	0.16	0.37	0.40	0.60
Control value (log) in WSH	6.33	6.30	6.13	6.10

Testing suitability for hygienic hand disinfection to EN 1500

Test organism: Escherichia coli NTCC 10538

Microbial count of baseline suspension: 9.48 log / ml

Inactivation substances:

3 % Tween + 3 % saponin + 0.1 % histidine + 0.1 % cysteine

Reference procedure:

Rub 2 x 3 ml 60 % (v/v) propan-2-ol for 60 sec

Test procedure:

Rub 3 ml "MANORAPID SYNERGY" on the hands for 30 seconds.

Test procedure: MANORAPID SYNERGY  
-original list -

- 30 sec

Test person	Hand	Prevalues			Postvalues		
		$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^0$	$10^{-1}$	$10^{-2}$
1	left	uc	150*	38*	uc	155*	15
	right	uc	285*	36*	uc	200*	39*
2	left	uc	260*	31*	uc	87*	11
	right	uc	315	38*	700	60*	8
3	left	uc	370	43*	400	43*	5
	right	uc	240*	28	uc	80*	14
4	left	uc	180*	33*	uc	83*	8
	right	uc	166*	31*	uc	110*	15
5	left	uc	360	70*	uc	212*	32
	right	uc	160*	22	uc	186*	20
6	left	uc	280*	38*	700	60*	5
	right	uc	250*	35*	uc	84*	7
7	left	uc	230*	41*	uc	240*	37*
	right	uc	225*	52*	uc	278*	29
8	left	uc	174*	31*	uc	102*	15
	right	uc	290*	28	uc	96*	14
9	left	uc	uc	114*	uc	700	86*
	right	uc	520	120*	uc	700	71*
10	left	uc	uc	135*	uc	uc	116*
	right	uc	uc	105*	uc	uc	130*
11	left	uc	730	90*	uc	143*	18
	right	uc	uc	105*	uc	162*	16
12	left	uc	124*	17	500	41*	6
	right	uc	105*	18	uc	85*	11
13	left	uc	280*	32*	253*	30*	6
	right	uc	440	47*	700	83*	14
14	left	uc	420	67*	uc	108*	12
	right	uc	460	76*	600	54*	7
15	left	uc	80*	12	68*	9	0
	right	uc	48*	7	53*	4	0

Reference procedure: 60 % iso-propanol - original list -

Test person	Hand	Prevalues			Postvalues		
		10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>0</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>
1	left	uc	170*	20	45*	2	0
	right	uc	250*	25	113*	6	0
2	left	uc	270*	43*	500	40*	3
	right	uc	320	47*	uc	81*	7
3	left	uc	440	36*	uc	120*	10
	right	uc	520	57*	uc	150*	17
4	left	uc	400	88*	450	50*	8
	right	uc	355	67*	224*	17	0
5	left	uc	173*	15	3*	0	0
	right	uc	214*	26	60*	9	0
6	left	uc	240*	28	24*	1	0
	right	uc	290*	31*	58*	9	1
7	left	uc	112*	14	uc	200*	30*
	right	uc	143*	17	uc	240*	26
8	left	uc	750	84*	uc	92*	12
	right	uc	680	73*	uc	184*	29
9	left	uc	uc	136*	uc	650	62*
	right	uc	uc	123*	uc	388	47*
10	left	uc	uc	108*	uc	uc	142*
	right	uc	865	113*	uc	uc	124*
11	left	uc	400	53*	uc	295*	31*
	right	uc	500	45*	uc	212*	17
12	left	uc	111*	18	uc	95*	11
	right	uc	96*	6	550	61*	10
13	left	uc	156*	17	500	47*	3
	right	uc	450	34*	800	72*	8
14	left	uc	530	45*	600	63*	9
	right	uc	340	51*	uc	84*	11
15	left	uc	215*	18	23*	5	0
	right	uc	145*	13	32*	3	0

\* = values selected for further calculation    uc = uncountable (≥1000)

MANORAPID SYNERGY							Reference procedure				
		logPV		logNV		logRF	logPV		logNV		logRF
1	le	6.098		3.190			6.230		1.653		
	ri	6.465	6.282	3.337	3.264	3.018	6.398	6.314	2.053	1.853	4.461
2	le	6.422		2.940			6.454		2.602		
	ri	6.580	6.501	2.778	2.859	3.642	6.672	6.563	2.903	2.753	3.810
3	le	6.633		2.633			6.556		3.079		
	ri	6.380	6.507	2.903	2.768	3.739	6.756	6.656	3.176	3.128	3.528
4	le	6.287		2.919			6.945		2.699		
	ri	6.253	6.270	3.041	2.980	3.290	6.826	6.886	2.350	2.525	4.361
5	le	6.845		3.346			6.238		0.477		
	ri	6.204	6.525	3.270	3.308	3.217	6.330	6.284	1.778	1.128	5.156
6	le	6.461		2.778			6.380		1.380		
	ri	6.413	6.437	2.924	2.851	3.586	6.465	6.423	1.763	1.572	4.851
7	le	6.392		3.401			6.049		3.320		
	ri	6.401	6.397	3.444	3.423	2.974	6.155	6.102	3.380	3.350	2.752
8	le	6.270		3.009			6.924		2.964		
	ri	6.462	6.366	2.982	2.996	3.370	6.863	6.894	3.265	3.115	3.779
9	le	7.057		3.935			7.134		3.792		
	ri	7.079	7.068	3.851	3.893	3.175	7.090	7.112	3.672	3.732	3.380
10	le	7.130		4.064			7.033		4.152		
	ri	7.021	7.076	4.114	4.089	2.979	7.053	7.043	4.093	4.123	2.920
11	le	6.954		3.155			6.724		3.472		
	ri	7.021	6.988	3.210	3.183	3.805	6.653	6.689	3.326	3.393	3.290
12	le	6.093		2.613			6.045		2.978		
	ri	6.021	6.057	2.929	2.771	3.286	5.982	6.014	2.785	2.882	3.132
13	le	6.452		2.410			6.193		2.672		
	ri	6.672	6.562	2.919	2.665	3.897	6.532	6.363	2.857	2.765	3.598
14	le	6.826		3.033			6.653		2.799		
	ri	6.881	6.854	2.732	2.883	3.971	6.708	6.681	2.924	2.862	3.819
15	le	5.903		1.833			6.332		1.362		
	ri	5.681	5.792	1.724	1.779	4.013	6.161	6.247	1.505	1.563	4.684
x			6.512	-		3.464		6.551	-		3.835
s			0.351	-		0.353		0.326	-		0.699

Paired statistical comparison of reduction factors obtained for the reference procedure and test procedure (WILCOXON matched pairs signed rank test)

	log RF MANORAPID SYNERGY	log RF Reference Procedure	Difference	Signed Rank
1	3.018	4.461	- 1.443	- 14
2	3.642	3.810	- 0.168	- 4
3	3.739	3.528	0.211	6
4	3.290	4.361	- 1.071	- 12
5	3.217	5.156	- 1.939	- 15
6	3.586	4.851	- 1.265	- 13
7	2.974	2.752	0.222	7
8	3.370	3.779	- 0.409	- 9
9	3.175	3.380	- 0.205	- 5
10	2.979	2.920	0.059	1
11	3.805	3.290	0.515	10
12	3.286	3.132	0.154	3
13	3.897	3.598	0.299	8
14	3.971	3.819	0.152	2
15	4.013	4.684	- 0.671	- 11
			Rank sum (-)	83
			(+)	37
			Significance level =	36
				( $p = 0.1$ )

The smaller rank sum (37) is below the significance level, therefore there is no significant difference between the two procedures.

### Results of the in vitro tests :

The product MANORAPID SYNERGY proved to be a bacteriostatic and fungistatic as well as a bactericidal und fungicidal product.

In the unchallenged qualitative suspension test, no test organisms could be cultured after exposure to a 50 % concentration for 30 seconds.

In the quantitative suspension test, a 50 % concentration achieved within 30 seconds a reduction of more than 5 log units of the test organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa* with and without albumin challenge.

### Results of the tests under everyday conditions

Suitability of MANORAPID SYNERGY for hygienic hand disinfection was investigated as per EN 1500. Comparison of the log reduction factors of the test and reference procedures shows a larger mean value of 3.84 log units for the reference procedure.

However, statistical comparison showed no significant difference between the test and reference procedure.

### Application recommendations

**Hygienic hand disinfection:**

**Rub 3 ml undiluted MANORAPID SYNERGY on the hands**

**Exposure time : 30 seconds**

(signature)

Prof. Dr. med. H.-P. Werner